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More accurate chronological age determination of crustaceans from field situations using the physiological age marker, lipofuscin

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Abstract A captive population of Australian red-claw crayfish, *Cherax quadricarinatus,* of known age was used for the study. Lipofuscin concentration in the left olfactory lobe cell mass of the brain was measured using image analysis of fluorescence micrographs. Age predictions based on lipofuscin concentration were more accurate and had narrower 95% confidence limits than those derived from the more conventional body size and weight predictors for all age groups tested (180, 300, 420, 780 d). Overall, use of lipofuscin concentration produced a significantly lower ($p< 0.001$) mean age-prediction error (16.65%) than use of carapace length or body weight (32.45 and 32.3%, respectively). Carapace length was of little value for prediction of the age of older individuals. Mathematical models defining the relationships between temperature, age and lipofuscin accumulation were derived from laboratory-reared individuals. These models did not describe adequately the course of lipofuscin accumulation in crayfish from the field over the whole lifespan. Field data from three "known" year-classes were used to generate simulated size-frequency and lipofuscin concentration-frequency histograms. Year-classes in the lipofuscin concentration frequency-histogram were much more easily distinguished than in the size-frequency histogram. Under field conditions, lipofuscin concentration was a better predictor of chronological age than conventional morphometric measures.

Introduction

Establishment of new age-estimation methods for use with crustaceans has been motivated largely by the need for more accurate ageing of species of commercial importance. The ability to determine numbers and sizes of individuals within particular age groups facilitates modelling of population dynamics, and hence more reliable catch forecasting and fishery management.

Lipofuscin deposits are irregular yellow-fluorescing granules found in the post-mitotic tissues of senescing animals. The structure of the main fluorophore in lipofuscin (from the human retinal pigment epithelium) has been identified recently as a lysosomotropic quaternary amine emitting at orange wavelengths (Eldred and Lasky 1993).

Lipofuscin can be detected using fluorescence microscopy and quantified with image analysis. When assayed in this way, lipofuscin concentration in the brain's olfactory lobe cell mass is a better predictor of chronological age in certain crustaceans, reared under controlled laboratory conditions, than is the conventionally-used body size relationship (Sheehy 1990 a, 1992). But what of its utility as an age index under field conditions? Natural environmental complexity and variability might affect lipofuscin accumulation in ways not observed in the laboratory. The primary objective of the present study was to test whether lipofuscin was a better predictor of chronological age than body size under temporally heterogeneous field conditions. Previous preliminary field results were encouraging (Sheehy 1992).

Laboratory-derived mathematical models relating temperature and age to pteridine age-pigment accumulation have proved very useful for age prediction of certain dipterans of agricultural and medical importance in the wild, under a range of conditions (e.g. Mail et al. 1983; Lehane and Mail 1985; Lehane et al. 1986; Lehane and Hargrove 1988; Msangi and Lehane 1991). Following earlier positive indications (Sheehy 1990 b), in this study we evaluated more fully the possibility of applying such modelling techniques to lipofuscin-based ageing of crustaceans from the wild.

Finally, we examined discrimination of population year-classes from lipofuscin concentration-frequency histograms, which was the original objective of Ettershank's (1983, 1985) pioneering work on krill.

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Materials and methods

Laboratory rearing

Approximately 2500 newly released crayfish, *Cherax quadricarinatus*, were collected from brooding females. These known-age crayfish were reared in the laboratory, at initial densities of 15 to 50 per container, in 20- to 45-litre plastic culture bins each lined with fine gravel and with aeration-driven under-gravel filtration. Short lengths of plastic tubing or nylon mesh were placed in the containers to act as shelters. The crayfish were fed daily ad libitum on crayfish feed pellets (35% protein). To maximize initial survival, water temperatures were held at a constant 23°C±2°C for 2 mo, then sub-groups of crayfish were reared for up to a further 800 d at constant temperatures of either 13, 18, 23, 28 or 33°C.

Field rearing

A single brood of *Cherax quadricarinatus* was reared in the laboratory at 23°C for 2 mo. Approximately 300 juveniles were then identified by clipping their telsons and placed 30/cage in floating cages in a 400 m^2 outdoor pond. Cages were 1×1×0.5 m, screened with 1 mm nylon mesh and supplied with short lengths of plastic pipe as crayfish shelter. All crayfish were tagged permanently after a further 2 mo by injection of a small piece of coloured nylon into the ventral abdominal tail muscle. Food pellets (35% protein), or fresh crustacean or fish pieces were added to the cages every 2 to 3 d. A rich epibiotic growth was also available on the cage walls for the crayfish to graze on. Waste detrital material fell through the mesh floor and larger pieces of left-over food and dead crayfish were removed prior to feeding. In the second year of the study, surviving crayfish were relocated, for security reasons, to individual holding containers in a partially shaded area of a building roof-top.

Data collection

In the field/outdoor rearing location, daily average water temperatures were monitored, either by the manual reading of maximum–minimum thermometers or by hourly measurements recorded on a data logger. At selected intervals throughout the course of the study, samples of 8 to 10 crayfish of mixed sex, representing the range of body sizes present in the experimental individuals, were sampled from the field rearing situation, and from each of the constant-temperature laboratory cultures. To define an individual's body size, its carapace length and wet whole weight were recorded. Lipofuscin concentration, as a percentage volume fraction (VF), was measured in the left olfactory lobe cell-mass of the brain using fluorescence microscopy and image analysis techniques (Sheehy 1990 b).

Mathematical treatment

In order to make valid comparisons between the utility of the three variables (lipofuscin concentration; carapace length; body weight) for predicting chronological age, the best-fitting mathematical models describing these relationships were determined. All regression models passed through the mean value of the dependent variable at each age group and therefore yielded minimum residuals. Any errors in age predictions therefore arose only from the inherent variability of the predictor, rather than poor fitting of the models. Since conditions were constant and experimental procedures did not commence until Age 60 d for all crayfish, this period was omitted from model fitting. All samples were tested for significant differences between males and females using a Student's *t*-test. Variability was compared between samples using a coefficient of variation adjusted for sample size, *V* (Sokal and Rohlf 1981; p. 59), after subtracting average starting values (at Age 60 d) from the data on each dependent variable.

A new descriptive parameter, the "resolution factor", was defined as the predicted age divided by its 95% confidence interval. Therefore, the higher the resolution factor, the finer the degree of age discrimination provided by the predictor variable.

Field data

Based on the graphical appearance of the plotted field data, and the knowledge that all three dependent variables were affected by temperature, seasonalized von Bertalanffy models (Hoenig and Choudary Hanumara 1990; Pauly et al. 1992) were applied to the data. The models were optimally fitted using iterative least-squares methods of parameter estimation and Sigma Plot 5.01 curve-fitting software (Jandel Corporation). The models were then used to predict ages of individuals by back-calculation. This involved iteratively varying the age term of the equation to achieve the observed value of the dependent variable. Predicted age was compared with known age, giving an estimate of age prediction error as a percentage of total age. Individuals from four age groups, 180, 300, 420 and 780 d, were assessed in this way and mean age prediction errors (MAPE) were determined for each age group and overall, for each of the three predictor variables. A Student's *t*-test was used to assess differences in the overall MAPE for each variable. Confidence intervals for inverse predictions of the independent variable, age, from each of the three dependent variables, were obtained by first linearizing the relationships using transformations of age. The appropriate transformations were obtained by reorganization of the respective seasonalized von Bertalanffy equations. Confidence limits for a predicted transformed age were then obtained using the formula of Sokal and Rohlf (1981; p. 498) for linear models.

Due to the differing variances of each sample age group, more accurate confidence intervals were obtained by splitting the transformed data set into four subsets, 60 to 180 d, 180 to 300 d, 300 to 420 d and 420 to 780 d, each containing two age groups. Separate linear regression parameters and statistics were determined for each subset and confidence limits were calculated for transformed ages, predicted from the mean dependent variable value for each age-group sample. Except for the youngest and oldest extremes, the confidence interval for each predicted age was therefore composite, with the upper limit being derived from regression parameters and statistics reflecting the variance of the data for individuals just older than the predicted age, and the lower confidence interval being derived from different regression parameters and statistics reflecting the variance of the data for individuals just younger than the predicted age. Transformed predicted ages and their confidence limits were de-transformed, again by iterative methods.

The age-predictive power of all three variables in combination was tested by applying the appropriate linearizing transformation to each predictor variable, then stepwise fitting a multiple linear regression. Coefficients of determination, R^2, were used to assess the contribution of each variable to predicting age.

Laboratory data

For each sample of laboratory-reared crayfish, the relationship between body weight and lipofuscin concentration was assessed with the product-moment correlation coefficient, *r*. Response surfaces for the laboratory data, relating age and temperature to either lipofuscin concentration, carapace length or body weight, were produced by non-linear interpolation between available sample means. In the case of 13 and 33°C temperature extremes, long-term survival of crayfish was poor. Response curves for older individuals at these temperatures were, therefore, partly inferred, their slope being based on the emerging trend for the early survivors in these regimes and their shape being based on that of the adjacent more complete 18 and 28°C data sets, respectively.

The relationships between temperature and each of the three inverse predictor variables were described by polynomial equations at all ages. Relationships between age and lipofuscin concentration at temperatures >18°C were best described by cubic von Bertalanffy functions. At and below 18°C, the relationships between age and lip-

ofuscin concentration were described by sigmoid functions. The relationships between age and carapace length were described by sigmoid functions at all temperatures, and age vs body weight was described by cubic von Bertalanffy functions.

A response matrix was produced based on these models. This matrix provided, at each 0.25 C° interval between 13 and 33°C, the expected lipofuscin concentration of crayfish for every day up to 900 d of age. Daily lipofuscin increments were calculated by subtracting the previous day's lipofuscin concentration from the current one. A BASIC program was written to read, from the response matrix, the appropriate daily lipofuscin accumulation rate resulting from the average field temperature (to the nearest 0.25 C°) for each experimental day/age. A predicted lipofuscin accumulation curve was generated for crayfish in the field situation by summing these daily increments over the period of the experiment. Expected accumulation was compared with actual accumulation.

Frequency-histogram simulations

Carapace length and lipofuscin concentration means and standard deviations for field crayfish from three age groups, 60, 420 and 780 d, were used to represent data for three hypothetical year-classes, 0+, 1+ and 2+. These statistics were used to generate hypothetical size-frequency and lipofuscin concentration-frequency histograms in which sample size was 350, annual mortality was 50%, and class interval was 1/40 of the range. For each age group, a normal distribution was generated using the normal probability density function (Sokal and Rohlf 1981, p. 111). The normal distributions for each age group were combined to form a frequency histogram. Separation of year class modes in each histogram was quantified using a separation index (Hasselblad 1966; Sparre et al. 1989).

Results

Sexual differences

Of the 20 temperature/age group combinations sampled from laboratory and field individuals of *Cherax quadricarinatus* during the study, 17 groups contained sufficient numbers of both males and females to permit *t*-test comparison of means between sexes. Only one of these 17 combinations, that at Age 180 d and 23°C, showed a significant difference in the mean lipofuscin concentration between males and females ($p < 0.05$). Two samples showed significant differences ($p < 0.05$) in mean carapace lengths between the sexes, 60 d/23°C and 300 d/18°C. Body weight differed significantly ($p < 0.01$) between the sexes in only one sample, 300 d/18°C. Since no consistent sexual differences were apparent in any of the variables measured, the sexes were not treated separately in further analyses.

Mathematical attributes of field data

Daily mean water temperature at the field site averaged 22.5°C overall, and ranged from 14.4 to 30.0°C during the period of the experiment. The frequency distribution of daily water temperatures was bimodal, with temperatures around 18 and 24°C being most common. Average daily water temperature is plotted along with the relationships between crayfish age and olfactory lobe cell-mass lipofuscin concentration, carapace length or body weight in Fig. 1.

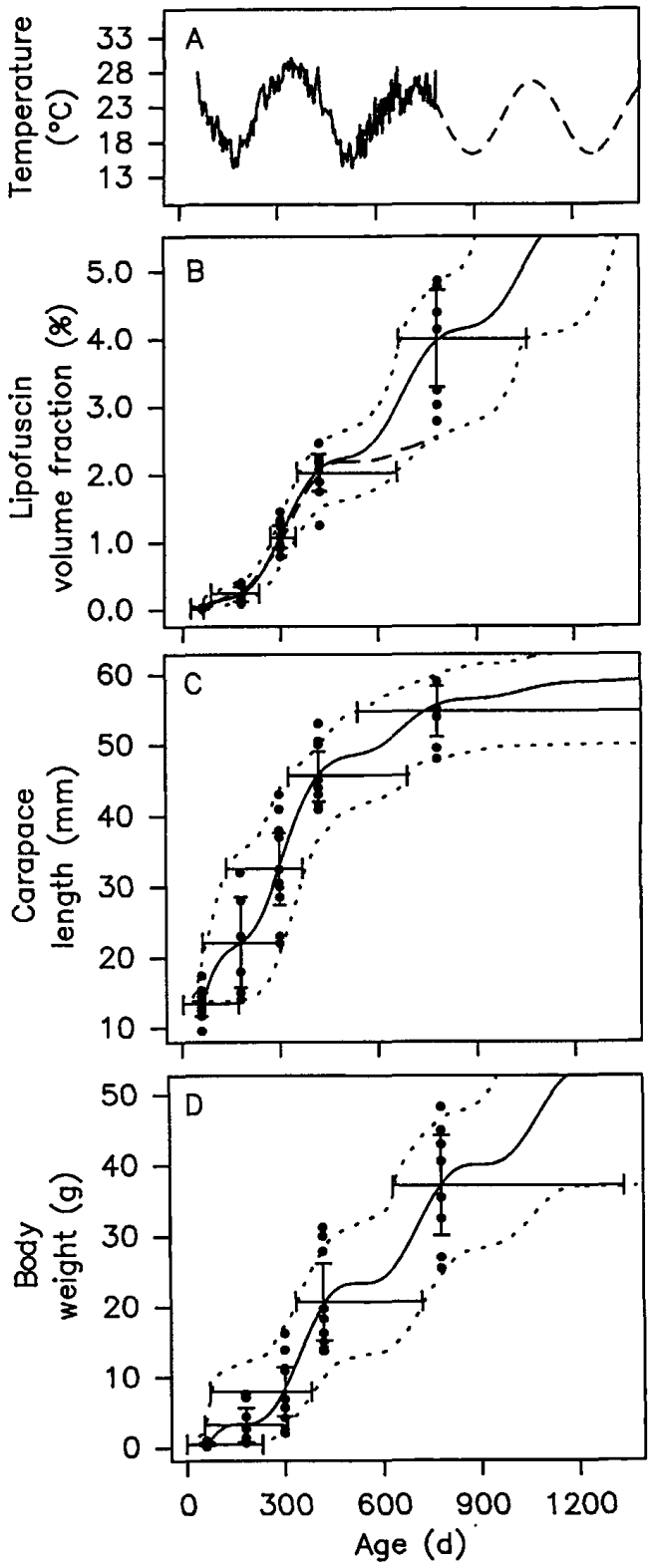

Fig. 1 *Cherax quadricarinatus*. Mean daily water temperatures at field site (**A**) and relationship between age and olfactory lobe cell-mass lipofuscin concentration (**B**), carapace length (**C**), and body weight (**D**) in experimental field population. Also shown are vertical 95% confidence limits for mean of each age group. Data are optimally fitted with seasonalized von Bertalanffy models (continous lines). Dashed portion of line in **A** represents predicted future average water temperatures, and in **B** predicted lipofuscin accumulation based on thermal-age models derived from laboratory data (*Dotted lines enclosing horizontal bars* 95% confidence limits for inverse prediction of ages from dependent variables; *confidence bands above oldest samples* putative only)

Table 1 *Cherax quadricarinatus.* Optimal parameter values for seasonalized von Bertalanffy models and adjusted coefficients of variation for field data. (Y_∞ asymptotic value of predictor variable; C amplitude of seasonal oscillation; K curvature factor; t_0 theoretical age at which predictor variable=0; t_s summer point or fraction of year which has passed before rate of increment in predictor variable is maximal; V adjusted coefficient of variation with subscript indicating age group)

Predictor variable	Seasonalized von Bertalanffy parameter					Adjusted coefficient of variation			
	Y_∞	C	K	t_0	t_s	V_{180d}	V_{300d}	V_{420d}	V_{780d}
Lipofuscin concentration	25.91	0.812	0.084	0.169	0.859	61.7	22.1	18.2	22.2
Carapace length	59.79	0.771	1.221	0.025	0.949	89.8	38.2	14.5	10.7
Body weight	124.7	1	0.179	0.160	0.960	108.2	67	36	23.7

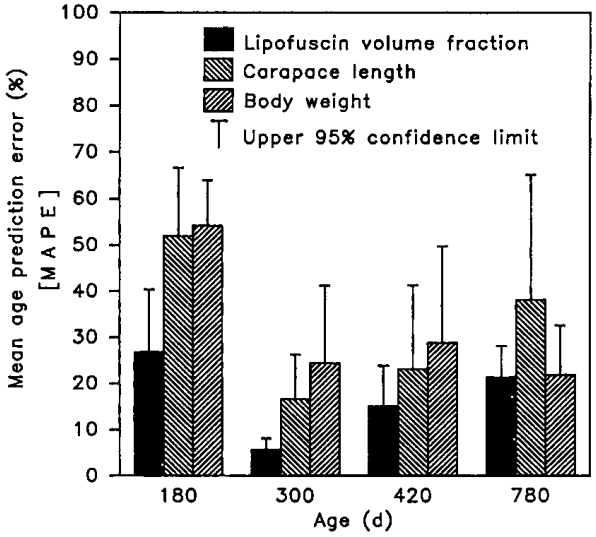

Fig. 2 *Cherax quadricarinatus.* Mean age prediction errors (MAPE) for experimental field crayfish of four age groups (180, 300, 420 and 780 d) after back-calculation from optimally fitted seasonalized von Bertalanffy models

Computer-optimized parameters for fitted seasonalized von Bertalanffy models are presented in Table 1. There was good agreement between summer points, t_s, in the three optimally fitted models, which corresponded to the period of highest temperatures in the field (December–February).

Adjusted coefficients of variation, V (Table 1), within the younger age groups, 180 and 300 d, indicated that there was less variation in lipofuscin concentration relative to rate of increment, than in either carapace length or body weight. In older age groups (420 and 780 d), V was greater for lipofuscin concentration than for carapace length, however rate of length increment was less than rate of lipofuscin accumulation, as indicated by the much greater curvature factor, K, of the age vs carapace length relationship. The age vs body weight relationship had both higher variability and slightly higher curvature than the relationship between age and lipofuscin concentration.

Age prediction by back-calculation from field data

Fig. 2 summarizes the resulting errors associated with age predictions based on the lipofuscin concentrations, carapace lengths or body weights of the experimental field individuals, using the seasonalized von Bertalanffy equations defined above. For the four age groups tested, MAPE using lipofuscin concentration ranged from 5.7 to 26.8%; using carapace length from 16.6 to 51.9% and using weight from 21.8 to 54.2%. Lipofuscin was in all cases the most accurate predictor. There was no significant difference (Student's t=–0.079, p=0.94) in the overall MAPE arising from carapace length or body weight age predictions (32.5 and 32.3%, respectively). On the other hand, lipofuscin concentration produced a significantly different and lower error rate than both carapace length (t=–3.066, p=0.0031) and weight (t=–3.390, p=0.0012), being twice as accurate as an age predictor (overall MAPE 16.65%).

Table 2 shows confidence limits, confidence intervals and resolution factors for inverse predictions of age based on lipofuscin concentration, carapace length or body weight. For all predicted ages, lipofuscin concentration produced better, that is, narrower confidence intervals and a finer level of age discrimination or resolution than the other variables. Because of the variable but asymptotic nature of growth in carapace length of older individuals, the upper confidence limit for age predictions in this region was theoretically infinite.

Correlation coefficients for relationships between age and linearized lipofuscin concentration, carapace length and body weight were 0.97, 0.84 and 0.90, respectively. In a stepwise regression analysis, the explained variance, R^2, in the relationship between linearized lipofuscin concentration and age was 93%. Addition of transformed body weight data to the model explained only a further 1% of the variance. Addition of transformed carapace-length data did not explain any further variance.

Relationships between dependent variables and age and temperature in laboratory

Fig. 3A, B and C, respectively, depict the response surfaces for lipofuscin concentration, carapace length and body weight, in relation to age and temperature. In general, all three variables increased in value with increasing age and temperature, but these relationships were not linear. In the case of lipofuscin accumulation, increment rate was minimal ($< 14\times10^{-7}$% VF d^{-1}) at low temperatures ($\lesssim$23°C) in older individuals ($\gtrsim$400 d). Accumulation rate

Table 2 *Cherax quadricarinatus.* 95% Confidence limits and intervals, and resolution factors for age predictions back-calculated from seasonalized von Bertalanffy models

Predicted age (d) and predictor variable	Predictor value	Lower 95% confidence limit (d)	Upper 95% confidence limit (d)	Confidence interval (d)	Resolution factor
180					
Lipofuscin conc. (%)	0.23	87	236	149	1.21
Carapace length (mm)	22.13	63	302	239	0.75
Body weight (g)	3.18	53	308	255	0.71
300					
Lipofuscin conc. (%)	1.06	269	346	77	3.9
Carapace length (mm)	19.21	133	372	239	1.26
Body weight (g)	7.46	72	380	308	0.97
420					
Lipofuscin conc. (%)	2.03	353	657	304	1.38
Carapace length (mm)	45.5	329	690	361	1.16
Body weight (g)	20.69	335	720	385	1.09
780					
Lipofuscin conc. (%)	4	661	1056	395	1.97
Carapace length (mm)	54.69	538	infinite	infinite	0
Body weight (g)	37.08	632	1339	707	1.1

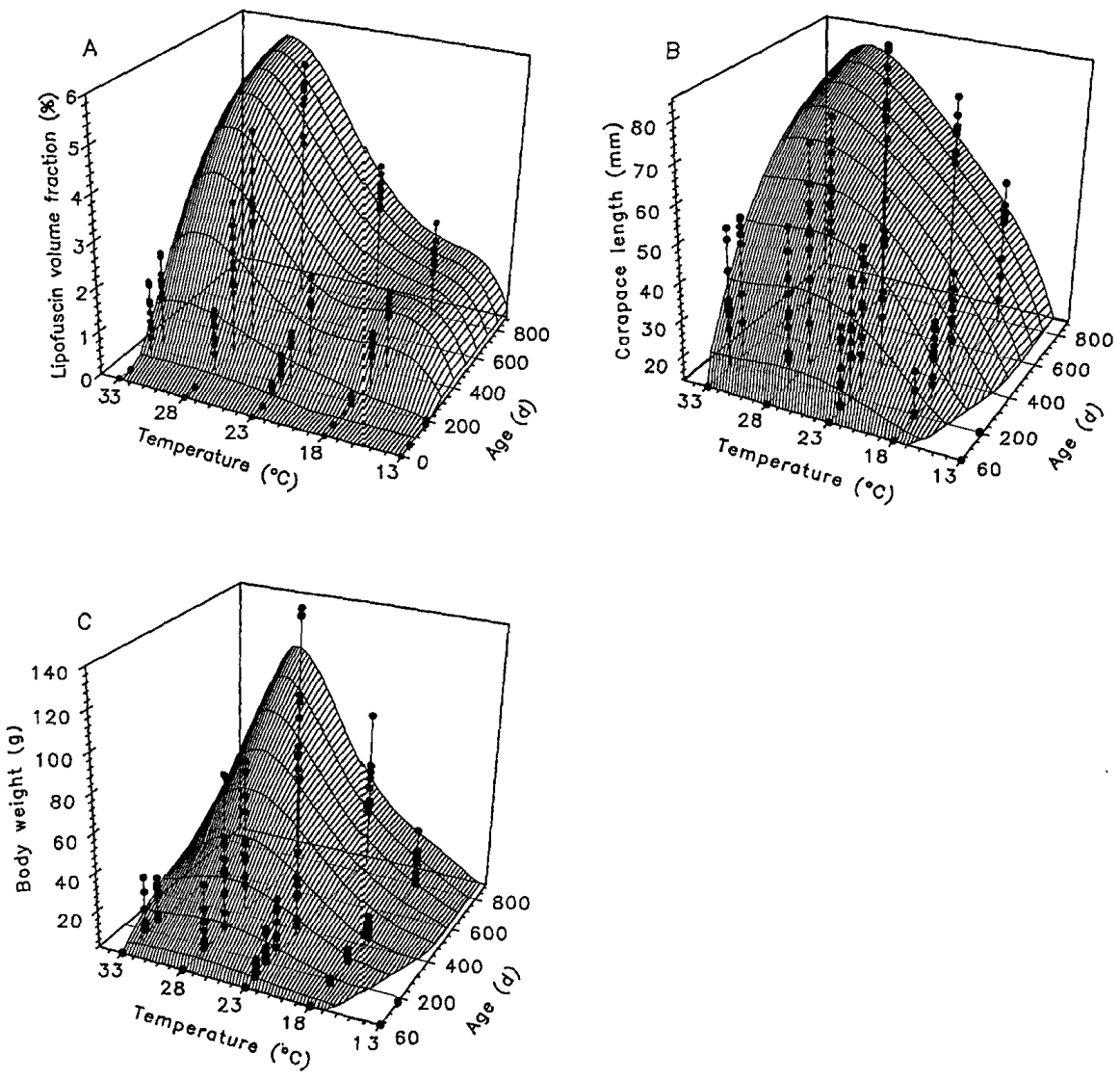

Fig. 3 *Cherax quadricarinatus.* Data points and interpolated response surfaces indicating relationships between environmental temperature, age and **A** olfactory lobe cell-mass lipofuscin concentration, **B** carapace length, and **C** body weight in laboratory-reared individuals

was maximal (0.011% VF d^{-1}) at higher temperatures (≳28°C) in younger individuals (≲400 d) and tended to decline with age at all temperatures. The rate of accumulation was relatively independent of temperature between 18 and 23°C and also levelled or declined above 28°C.

Survival was poor and growth appeared to be negative (av −0.012 mm d^{-1}) at 13°C. The minimum temperature necessary for positive growth of juveniles was ~17°C. Highest carapace growth rate (av 0.167 mm d^{-1}) occurred in crayfish up to ~200 d at temperatures between 23 and 33°C. Carapace growth rate declined with advancing age at all temperatures but, in older individuals, was highest at 28°C. Rate of weight gain was maximal (0.167 g d^{-1}) at ~28°C, with a distinct indication of a decline at higher temperatures.

As is apparent from Fig. 3, the laboratory data showed a similar trend to the field data in that the overall level of scatter of lipofuscin concentrations within age groups, relative to rate of increment, was lower than for carapace length or weight.

Predicted lipofuscin accumulation in field based on laboratory thermal-age models

The predicted lipofuscin accumulation curve for field individuals, based on the laboratory-generated thermal-age response matrix, is shown as a dashed line in Fig. 1B. As indicated by the vertical confidence limits on the data, this curve did not differ significantly ($p < 0.05$) from the actual lipofuscin accumulation curve up to at least 420 d. After this age the expected lipofuscin accumulation was markedly lower than the actual accumulation.

Relationship between lipofuscin concentration and body weight

In the 17 samples taken in the laboratory-rearing experiments, comprising a variety of temperature/age combinations, the correlation between lipofuscin concentration and body weight ranged from −0.66 to +0.78, with the mean absolute correlation being 0.39. There was no clear trend in the correlation between lipofuscin concentration and weight across the samples which could be related to temperature or age.

Year-class discrimination in simulated frequency-histograms

Separation of simulated 0+ and 1+ year-class modes was complete using either lipofuscin concentration or carapace length in frequency histograms (Fig. 4). Mean carapace length and standard deviation at Age 420 d were 45.5±4.51 mm and at 780 d, 54.69±4.28 mm. Based on carapace length, it was impossible to discriminate simulated 1+ and 2+ modes with a separation index of 2.09. On the other hand, mean lipofuscin concentration and standard deviation at 420 d were 2.026±0.358%; and at 780 d, 3.996±0.856%. It was readily possible to discriminate the simulated 1+ and 2+ year-class modes using lipofuscin with a separation index of 3.25.

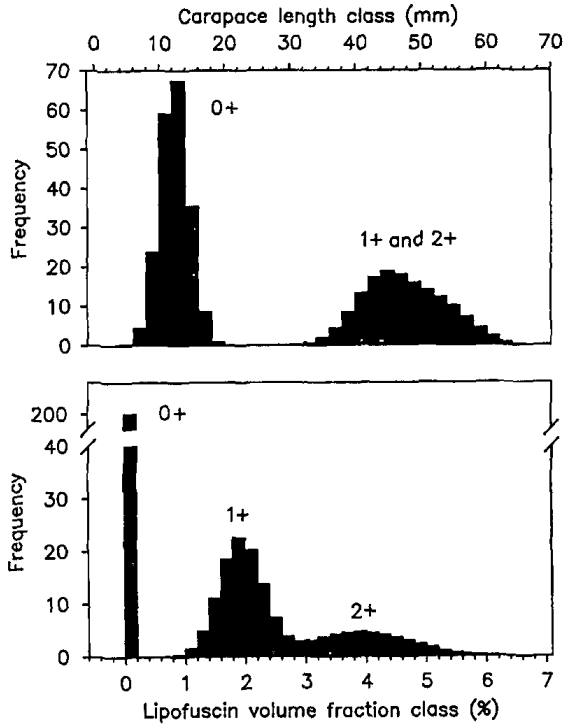

Fig. 4 *Cherax quadricarinatus.* Simulated lipofuscin concentration-frequency and carapace length-frequency histograms based on data from field-rearing study (*0+, 1+ and 2+* simulated year-class modes)

Discussion

By necessity, the scope of this discussion is confined to issues relating to the use of lipofuscin, carapace length and body weight as predictors of crustacean age. Areas pertaining particularly to the effect of temperature on lipofuscin accumulation and growth will be dealt with in more detail in separate papers.

The apparent lack of sexual difference in lipofuscin accumulation rate across a wide range of constant temperatures and in the field is consistent with earlier findings for *Cherax quadricarinatus* (Sheehy 1990 a) and also *C. cuspidatus* (Sheehy 1990 b). This feature conveniently removed the need for separate mathematical treatment of sexes for descriptive and predictive modelling. Sexual differences in individuals of identical age have generally not been reported from research utilizing reliable histological methods for lipofuscin quantification. However, sample sizes in the present study were small and may not have revealed slight sexual differences. As a precaution, this comparison should still be made for other crustacean species in any future studies.

Lipofuscin accumulation rate decelerated with advancing age and was profoundly affected by environmental tem-

perature over a lifespan covering several seasons. This necessitated the use of a mathematical model incorporating parameters defining both negative exponential increment and seasonal oscillation to describe field data. Seasonalized von Bertalanffy models were therefore appropriate and, although they are in fairly common use as fisheries growth curves, they have not been fitted previously to age-pigment data. There was very good agreement between all three models in the time of year of maximum increment rate and the highest environmental temperatures. Lipofuscin concentration was shown in this and earlier studies to be relatively independent of carapace length and weight in these crayfish (Sheehy 1990 a), so the convergence of summer points for the three models is good evidence that the data oscillations are real seasonal ones and that seasonalized von Bertalanffy equations are appropriate.

The field data continued to show two features characteristic of previous laboratory results (Sheehy 1992). Firstly, in younger age groups individual variability in lipofuscin concentration relative to rate of accumulation was less than that recorded for carapace length or weight. Secondly, in older individuals rate of increment was relatively greater for lipofuscin concentration than for carapace length or weight. The result of these attributes was that age groups were generally better distinguished by lipofuscin concentration than by the other two variables. Carapace length of mature crayfish in the field entering the asymptotic phase of their growth was virtually useless for accurate age prediction, since the upper 95% confidence limit for any such age estimate was theoretically infinite. These results confirmed for this species that, under field conditions, lipofuscin concentration continued to be a better predictor of chronological age than the conventionally used morphometric measures.

Another approach to age estimation which has been used successfully involves the development of thermal-age models which account for the overriding effect of environmental temperature on the rate of increment of the predictor variable. The development of valid thermal-age models may alleviate the need for separate mathematical descriptions of growth or age-pigment accumulation to be developed at each new thermal location (Mail et al. 1983; Stevens 1990).

It is now apparent that the expectations of simple linear relationships between temperature and age-pigment accumulation rate, which have occurred in the literature, may be unrealistic for crustaceans and other organisms. This is particularly likely to be the case when, as in the present study, temperatures encompassing most or all of the tolerance range of the species are examined. Thermal models for lipofuscin accumulation in the present study were relatively complex polynomial equations. This is precisely what is expected for a cellular feature which represents the "integral of oxidative metabolism over the lifespan" (Mullin and Brooks 1988). The relationship between temperature and physiological rate takes a sigmoid or polynomial form in many marine invertebrates (Newell 1979; Newell and Branch 1980; Zoutendyk 1989). This form reflects both thermal tolerance limits and acclimatization or compensation mechanisms which are well known attributes of animal metabolism (Prosser 1973).

The relationship between body weight and metabolic rate in crayfish (e.g. Villarreal 1990) and other animals is well documented. Since lipofuscin accumulation rate is also linked to metabolic rate, this raises the question as to whether a size or weight term needs to be added to any model incorporating lipofuscin as an age predictor. In an earlier study (Sheehy 1990 b), lipofuscin concentration appeared to be correlated with body size. This trend has not held true in the present study. Although correlations were occasionally relatively high within age groups, no consistent trends between lipofuscin concentration and body weight could be established.

Applying our laboratory-derived thermal age model resulted in a very close fit to actual field data for younger age groups, but a significant underestimation of lipofuscin concentrations in the oldest crayfish. This inaccuracy stemmed largely from the depressed (or acclimatized) accumulation rate in older laboratory-reared individuals, particularly those reared at 18°C, which reduced the predicted winter accumulation rate in the field to near zero. Probably the metabolic status of individuals reared in the laboratory for long periods under constant and unnatural conditions is not an adequate reflection of the field situation. A better approach may be to produce the necessary data by rearing individuals in open systems which have naturally fluctuating temperatures, but there will still be environmental differences between such captive situations and the field. Also, the size and longevity or very specific habitat or food requirements of many commercial species will preclude this type of large-scale experimental rearing from the start. Laboratory-based thermal-age modelling using pteridine age pigment as the predictor, has proved very successful for chronological age determination in certain short-lived insects (e.g. Mail et al. 1983). However, the present results suggest that for longer-lived crustaceans, thermal-age models describing age-pigment accumulation in laboratory-reared individuals may not be able to predict accurately the age of older individuals from the field by their age-pigment levels.

Whilst examining the effects of temporal environmental variation, this study has not directly tested the effect of spatial heterogeneity in temperature and other environmental variables on lipofuscin accumulation in field individuals. This is a possible area of difficulty for age-pigment-based age prediction. Where the geographical range of a species is large, the effect of temperature on lipofuscin accumulation rate may limit the transferability of a prediction model from one thermal regime to another. Exactly what spatial scales constitute "different" thermal regimes in terms of a significant effect on the lipofuscin accumulation curve are unknown, and are likely to vary with situation and species. Also, long-distance migration of animals through different thermal regimes may confound the issue. It is apparent from the present laboratory and field data that shifts in temperature through the full range experienced at the field site had a marked effect on the lipofuscin accumulation rate. It is also apparent from the labora-

tory-derived thermal-age response-surface that between 18 and 23°C, the lipofuscin accumulation rate is relatively independent of temperature. Interestingly, this flattened response occurs over the mean and modal water temperatures experienced at the field site. As noted earlier, this may well reflect an ability to metabolically acclimatize over the modal or preferred thermal range. It is possible that such compensations might simplify matters by tending to negate potential effects of moderate spatial environmental heterogeneity. Lipofuscin is now being measured in tagged, known-age, European lobster, *Homarus gammarus*, which were released into the wild around the coastline of Britain as juveniles in the mid 1980s (Wickins and Sheehy 1993; Sheehy and Wickins 1994; M. Belchier and P. M. S. Shelton personal communication). This study will help to resolve the question of the effect of spatial environmental heterogeneity on lipofuscin accumulation.

A further approach to the utilization of lipofuscin for crustacean age determination suggested by the results of this study may be a modification of length-frequency analysis, replacing measurement of the animal's length with its lipofuscin concentration. Modal analysis of fluorescent age-pigment concentration-frequency histograms has been attempted before (Ettershank 1983, 1985; Berman et al. 1989; Nicol et al. 1991), but fundamental problems with the available biochemical quantification methodology confound interpretation of these results. Scatter in sizes of individuals of the same year-class and declining growth rate with age pose a considerable problem in length-frequency analyses, since they result in the merging of modes and the inability to separate year-classes. This problem was exemplified in the simulations performed in the present study. Modes in the carapace length-frequency distribution arising from mature crayfish could not be separated, since carapace length hardly changed over the course of the second year. Likewise, distinguishing the 1+ and 2+ year-classes in real size-frequency histograms from natural populations is difficult (Jones 1990). As a general rule, if the separation index is < 2, it is virtually impossible to separate the components using even the most sophisticated computer techniques (Sparre et al. 1989; p. 119). On the other hand, there is less variance in lipofuscin concentrations than in body size in younger individuals of the same age and less deceleration in rate of increment in older individuals. Therefore, lipofuscin concentration may offer a completely new basic dimension by which year-classes can be separated. The usefulness of morphological lipofuscin for year-class discrimination in wild crustacean populations is presently being tested using the western rock lobster *Panulirus cygnus*. A positive result will have important implications for fisheries population age-structure analysis.

In conclusion, the results of this project have confirmed the potential of lipofuscin for age determination of a crustacean under field conditions and have clarified directions in which it may be most fruitful for future research to proceed. Further studies are now required on other species, under field conditions, to validate these results and establish the breadth of usefulness of lipofuscin for ageing crustaceans from the field.

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